APPENDIX C (cont.)

A comparison of Mehlhorn claims 28, 33-37, 39, and 44-50 in light of the Fendler Reference

Fendler, which was not before the PTO during prosecution of the Mehlhorn application, serves as a § 102(b) prior art reference as of its April 15, 1980 publication date. Fendler teaches that use of buffers allows maintenance of pH gradients for extended periods of time by reducing proton permeability through the liposome membrane, thereby increasing entrapment efficiency and reducing leakage. (See Prestegard Declaration, ¶ 15, FR vol II, tab 8 at 19). Fendler discusses data showing (1) maintenance of pH gradients in excess of 2 units across liposomes for a period of several hours or longer; and (2) that proton permeability across the lipid bilayer of liposomes depends upon the buffer used to adjust the pH inside the liposome. (See Prestegard Declaration, ¶ 15, FR vol II, tab 8 at 19). Fendler specifically states that:

> These data provide means for the more effective entrapment of drugs. It should also be possible to preserve sensitive drugs in the carriers at pH values different from the surrounding in vivo media.

FR Exh 7, Fendler at 96.

Claim Chart III

Claims 28, 33-37, 39 and 44-50

Mehlhorn	Cramer in view of Fendler or Nichols
28. The method of Claim 27, wherein in (a) (i) an acidic liposome-containing aqueous medium is formed in which the acid is present in both the internal and external liposome phases, and in (c) (i) a base is added to the external phase to thereby induce the cationic chemical species to pass into the liposomes' internal aqueous phase.	Cramer teaches forming liposomes from egg PC and cholesterol in aqueous maleate solution; maleate was present in both the internal and external liposome phases [MATERIALS & METHODS, p. 296, lines 1-3 and p. 297, lines 1-5], and adding deuterated hydrochloric acid to the external liposome phase to cause fumarate to pass into the liposomes' internal aqueous phase [MATERIALS & METHODS, p. 297, lines 6-10]; Mehlhorn admitted that alternative (i), i.e., forming an acidic liposome-containing aqueous medium, and adding a base to induce a cationic species to pass into the liposomes' internal phase would have been obvious over alternative (ii), i.e., forming a basic liposome-containing aqueous medium, and adding an acid to induce an anionic species to pass into the liposomes' internal phase [Reply to Restriction Requirement, Paper No. 13, p. 2, lines 5-9; Amendment and Renewed Request for Interference, Paper No. 16, pp. 3-4].
31. The method of Claim 27, wherein the base which is added to thereby induce the cationic species to pass into the liposomes' internal aqueous phase in (c) (i), or the acid which is added to thereby induce the anionic chemical species to pass into the liposomes' internal aqueous phase in (c) (ii) is a component of a buffer.	Fendler teaches that using the proper buffers allows maintenance of pH gradients for extended periods by reducing adventitious proton transport through the lipid membrane, whereby increasing entrapment efficiency and reducing leakage [p. 96, lines 5-9; 20-27].

32. The method of Claim 30 wherein the base which is added to thereby induce the cationic species to pass into the liposomes' internal aqueous phase in (c) (i), or the acid which is added to thereby induce the anionic chemical species to pass into the liposomes' internal aqueous phase in (c) (ii), is a component of a buffer.	Fendler teaches that using the proper buffers allows maintenance of pH gradients for extended periods by reducing adventitious proton transport through the lipid membrane, thereby increasing entrapment efficiency and reducing leakage [p. 96, lines 5-9; 20-27].
33. The method of Claim 27, wherein the charged chemical species is a drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH to ionize available functional groups on the drug [p. 96, lines 5-9; 20-27].
34. The method of Claim 27 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.
35. The method of Claim 30 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.
36. The method of Claim 30 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.

37. The method of Claim 32 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.
39. The method of Claim 38, wherein in (a) (i) an acidic liposome-containing aqueous medium is formed in which the acid is present in both the internal and external liposome phases, and in (c) (i) a base is added to the external phase to thereby induce the cationic chemical species to pass into the liposomes' internal aqueous phase.	Cramer teaches forming liposomes from egg PC and cholesterol in aqueous maleate solution; maleate was present in both the internal and external liposome phases [MATERIALS & METHODS, p. 296, lines 1-3 and p. 297, lines 1-5], and adding deuterated hydrochloric acid to the external liposome phase to cause fumarate to pass into the liposomes' internal aqueous phase [MATERIALS & METHODS, p. 297, lines 6-10]; Mehlhorn admitted that alternative (i), i.e., forming an acidic liposome-containing aqueous medium, and adding a base to induce a cationic species to pass into the liposomes' internal phase would have been obvious over alternative (ii), i.e., forming an basic liposome-containing aqueous medium, and adding an acid to induce an anionic species to pass into the liposomes' internal phase [Reply to Restriction Requirement, Paper No. 13, p. 2, lines 5-9; Amendment and Renewed Request for Interference, Paper No. 16, pp. 3-4].
42. The method of Claim 38, wherein the base which is added to thereby induce the cationic chemical species to pass into the liposomes' internal aqueous phase in (c) (i), or the acid which is added to thereby induce the anionic chemical species to pass into the liposomes' internal aqueous phase in (c) (ii), is a component of a buffer.	Fendler teaches that using the proper buffers allows maintenance of pH gradients for extended periods by reducing adventitious proton transport through the lipid membrane, thereby increasing entrapment efficiency and reducing leakage [p. 96, lines 5-9; 20-27].

43. The method of Claim 41 wherein the base which is added to thereby induce the cationic chemical species to pass into the liposomes' internal aqueous phase in (c) (i), or the acid which is added to thereby induce the anionic chemical species to pass into the liposomes' internal aqueous phase in (c) (ii), is a component of a buffer.	Fendler teaches that using the proper buffers allows maintenance of pH gradients for extended periods by reducing adventitious proton transport through the lipid membrane, thereby increasing entrapment efficiency and reducing leakage [p. 96, lines 5-9; 20-27].
44. The method of Claim 38 wherein the charged chemical species is a drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH to ionize available functional groups on the drug [p. 96, lines 5-9; 20-27].
45. The method of Claim 38 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.
46. The method of Claim 41 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.
47. The method of Claim 42 wherein the charged chemical species is a hydrophobic drug.	Fendler teaches that enhanced entrapment of drugs in liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.

48. The method of Claim 43 wherein the charged chemical species is a hydrophobic drug.

Fendler teaches that enhanced entrapment of drugs I liposomes can be achieved by adjusting pH [p. 96, lines 5-9; 20-27]; drugs which can accumulate inside a liposome in response to a pH gradient are "relatively lipophilic" as evidenced by their ability to pass through the lipid membrane.

49. The method of Claim 38 wherein said aqueous medium containing an acid in step (a) (i) has a pH less than 7 and wherein said aqueous medium containing a base in step (a) (ii) has a pH greater than 7.

Mehlhorn admitted that the particular pH of the aqueous medium was immaterial so long as a pH gradient was established [March 22, 1993 Amendment, Paper No. 9, p. 6];

Nichols teaches forming liposomes in an aqueous citrate-phosphate buffer at pH 5 [p. 270, lines 5-7].

50. The method of Claim 49 wherein said aqueous medium containing an acid in step (a) (i) has a pH of 5.0 and wherein said base added to the external liposome phase in step (c) (i) raises the pH of the external liposome phase to 7.4.

Mehlhorn admitted that alternative (i), i.e., forming an acidic liposome-containing aqueous medium, and adding a base to induce a cationic species to pass into the liposomes' internal phase would have been obvious over alternative (ii), i.e., forming an basic liposome-containing aqueous medium, and adding an acid to induce an anionic species to pass into the liposomes' internal phase [Reply to Restriction Requirement, Paper No. 13, p. 2, lines 5-9; Amendment and Renewed Request for Interference, Paper No. 16, pp. 3-4];

Mehlhorn further admitted that the particular pH of the aqueous medium was immaterial so long as a pH gradient was established [March 22, 1993 Amendment, Paper No. 9, p. 6];

Nichols teaches forming liposomes in an aqueous citrate-phosphate buffer at pH 5 and adding sufficient base to raise the pH of the external aqueous medium to 8 [p. 270, lines 5-12]. There is no qualitative difference in the choice of a pH of 8 versus 7.4 in this application.